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# Molecular Effects of Targeting Multidrug Resistance of Doxorubicin Treated Breast Cancer Cells Using the Calcium Channel Blocker Nimodipine

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**Abstract**

Background/Aim: Multidrug resistance (MDR) is the leading cause of treatment failure in breast cancer patients treated with doxorubicin DOX). Previous indicated that some calcium channel blockers could reverse multidrug resistance. Therefore, we aimed to investigate the potential of nimodipine (a calcium channel blocker drug) in sensitizing breast cancer cells to DOX and elucidate the underlying molecular mechanisms. Methods: we examined the effects of DOX alone or in combination with nimodipine (NMD) on the viability of the MCF-7 cells using MTT assay, cell cycle by flow cytometry, and the expression of the MDR-related gene (MDR1) and cell cycle/survival gene (Bcl-2) and the pro-apoptotic gene (Bax) by quantitative reverse transcription polymerase chain reaction. Results: we found that adding NMD to DOX potentiated its antiproliferation effect. The value of the combination index (CI) of NMD/DOX was less than 1 indicating a synergistic effect. Combined DOX/NMD treatment also caused G1 arrest and potentiated apoptosis more than DOX-single treatment. At the molecular levels, NMD/DOX treatment downregulated the mRNA of MDR1 and Bcl-2; while upregulated the Bax gene compared with DOX alone. Conclusion: the results confirmed the potential of NMD in sensitizing Breast cancer to DOX by targeting MDR1 and suppressing the Bcl-2 gene while upregulating the Bax gene. Additionally, NMD could be repurposed to reduce the therapeutic doses of DOX as indicated by the dose reduction index (DRI) and subsequently decrease its side effects (especially cardiotoxicity), along with decreasing the chemoresistance of breast cancer cells to DOX treatment.

*Keywords*: Apoptosis, calcium channel blocker, cell cycle, chemoresistance, doxorubicin, nimodipine.

## 1. Introduction

Cancer is a diverse category of diseases characterized by uncontrolled cell growth that represents the greatest cause of mortality and morbidity worldwide. It is a long-term process that starts with a single mutation and builds up through the years to make the first out-of-control cell and thereafter the tumor as discussed by Watson (1). Breast cancer (BC) is one of the most common cancers worldwide that affects over 2 million women each year (2). BC is an invasive tumor that is considered the main cause of overall cancer-related deaths, especially in women aged between 35 and 75 years old (3,4) DOX is one of the most used chemotherapeutic agents, particularly in advanced or metastasis cancer patients. Mechanically, DOX represses topoisomerase II (Top II) and intercalates directly to DNA double-strand, finally, resulting in the intervention of gene transcription (5). Cardiotoxicity is the most important side effect of doxorubicin, (6) which is one of the most dangerous dose-limiting toxicities of this drug. Several studies have been conducted to find new strategies to maximize clinical efficacy while limiting side effects of doxorubicin (7). The second evident problem using Dox is the acquired tumor resistance against it (8). The development of chemotherapy resistance in Breast Cancer is mediated by multiple signaling pathways involving the induction of proliferation, cell cycle progression, and prevention of apoptosis (9). DOX drug resistance is developed as a result of increased expression of the ATP-dependent efflux pump ABCB1 (MDR1), (10) which encodes the membrane drug transporter P-glycoprotein and often contributes to poor prognosis and development of metastatic tumors resistant to chemotherapy such as BC (11). P-gp, also known as ATP-binding cassette subfamily B member

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1 (ABCB1), functions as a transmembrane ATP-dependent drug efflux pump for a variety of toxins, xenobiotics, and drugs (12). P-gp is encoded by the MDR1 gene, which is found upregulated in several BC subtypes causing a reduction in the intracellular accumulation of multiple chemotherapeutic drugs; including DOX; leading to multidrug resistance (MDR) (12). Recent studies have pointed out the importance of drug re-purposing and its potential in identifying novel therapeutic uses for already known drugs (10). Drug repurposing (or drug repositioning) is an accelerated tool for drug development that involves seeking new indications for drugs that are already FDA-approved rather than discovering new compounds and currently constitutes 30% of the newly marketed drugs in the United States (10,13). Many successfully repurposed drugs have been introduced into the market as in the case of aspirin for the treatment of stroke and/or myocardial infarction and topiramate for the treatment of obesity (14). Nimodipine (NMD), NMDP is a calcium channel blocker (CCB) belonging to the dihydropyridine class and is a highly lipophilic agent that rapidly crosses the blood-brain barrier (Figure 1). Its mechanism of action is the selective blockage of intracellular calcium ions influx through L-type VGCCs (15).

Calmodulin is a calcium-binding protein which is regulating many of the intracellular actions of calcium. It is proposed that calmodulin is responsible for the regulation of cellular proliferation and that its function may be altered in malignancy. Besides, calmodulin antagonists are cytotoxic and can restore the sensitivity of resistant cells to drugs such as DOX and vincristine (17) Therefore, we sought to investigate the efficacy of NMD to counteract the drug resistance of MCF-7 cell line against DOX and to elucidate the underlying mechanism at the molecular level. We report that treatment of Breast Cancer cells with NMD decreases the expression of the multidrug-related gene MDR1.

## 2. Materials And Methods

### *2.1. Chemicals and cells*

Nimodipine was obtained from Tocris Bioscience™; CAS, 66085-59-4 (Cat. No. 0600). Doxorubicin (MW = 543.5, purity > 98.0%, HPLC) was purchased from Sigma (cat. no. D1515, St. Louis, MO). RPMI 1640 medium and fetal bovine serum (FBS) were purchased from GIBCO (Invitrogen, CA). MCF-7 breast cancer cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS), 50 U/ml penicillin/streptomycin, and 2 mM l-glutamine in a humidified incubator at 37 °C and 5% carbon dioxide. Primers were obtained from (Applied Biosystems), RNA extraction kit obtained from (Qiagen, Hilden, Germany) and PCR kit HERA SYBER GREEN/ROX RT-PCR obtained from (Applied Biosystems, Foster City, California, USA). Real-time qPCR amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). Analysis of Quantitative DNA content in cultured cells was measured by Ab139418 DNA flow cytometry analysis Kit.

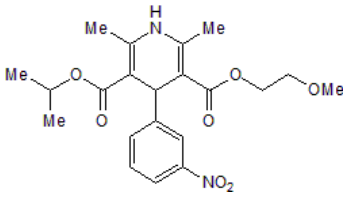


Figure 1: Chemical structure and formula of nimodipine. 3-(2-methoxyethyl) 5-propane-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate C21H26N2O7 (16).

### *2.2. MTT Cytotoxicity Assay:*

The cytotoxic activity of NMD was measured in vitro against the Breast cell line compared to DOX as a reference drug. MCF-7 cells were treated with DOX, NMD, or DOX combined with NMD, respectively, for 24 h at different concentrations. Multi-well plates were used in the MTT method, and the final number of cells should not exceed $10^6$ cells/cm2 in the log phase of growth for the best results. Also, untreated cells were included for each experiment as control cells. Cells were treated with NMD or DOX at different concentrations (from 100 to 0.39 uM) for 24 h, and the killing effect of different concentrations was recorded. The half-maximal inhibitory concentration (IC50) values were analyzed and used to determine the concentrations to be used in NMD/DOX combinations, comprising the ratio of IC50 DOX/IC50 NMD. The effects of the combination of NMD on the antitumor activity of DOX on MCF-7 cells were also recorded.

### *2.3. Drug combination Analysis.*

Drug combination studies were carried out using CompuSyn software version 1.0 (Ting Chao Chou and Nick Martin, Paramus, NJ). The combination index (CI) was measured based on the mass action law of the degree of drug interaction according to Chou and Talalay. CI calculation is based on the formula CI = (D)1/(Dx)1+(D)2/ (Dx)2, where (Dx)1 and (Dx)2 represent the doses of NMD and DOX in a combination which was required to achieve the same efficacy as that of NMD (D1) and DOX (D2) when used alone (18) CI < 1 indicates synergism, where CI = 1 indicates an additive effect and CI > 1 indicates

antagonism. Also, the drug reduction index (DRI) values above 1 imply a favorable dose reduction in the drug combination compared to the monotherapy.

*2.4. DNA-Cell cycle analysis by Flow Cytometry*

Cell cycle phases in samples of untreated or treated MCF-7 cell cultures were analyzed using flow cytometry as previously described (19). Briefly, MCF-7 breast cancer cells were seeded at $8 \times 10^4$ cells/well and incubated overnight at 37 °C, and supplied with 5% CO2. MCF-7 cells were treated by the IC50 of the three treatments (DOX, NMD, and NMD/ DOX combination), and their impact on the cell population was recorded and compared to the control (media). After 48 h of treatment, cell pellets were collected and centrifuged at 300g for 5 min. For cell cycle analysis, cell pellets were fixed in 70% ethanol on ice for 15 min. The collected pellets were incubated with propidium iodide (PI) staining solution (50 µg/mL PI, 0.1 mg/mL RNase A, and 0.05% Triton X-100) at room temperature for 1 h. Stained cells were kept in the dark at 4 °C until analysis using flow cytometry.

*2.5. Reverse transcription and quantitative real-time polymerase chain reaction (PCR)*

The MCF-7 cells were treated with DOX alone or in combination with NMD at concentrations of 0.39–100uM for 48 h were digested with trypsin-EDTA solution, centrifuged, and harvested. The total RNA was extracted from the cells using TRIzol reagent (Life Technologies, Inc.) as described by the manufacturer and reverse-transcribed into cDNA. The primer sequences for Bcl2, Bax, MDR1, and the housekeeping gene GAPDH were designed using the software Primer version 5.0 (Premier Corporation) (Table 1).

**Table 1: Primers Sequences of the Target Genes (Bcl2), (Bax), (MDR1), and the Housekeeping Gene (GAPDH)**

| Gene | Primer Sequence |
|---|---|
| Bcl2 | F 5′- TCGCCCTGTGGATGACTGA-3′R<br>R 5′-CAGAGACAGCCAGGAGAAATCA-3′R |
| Bax | F 5′-TGGCAGCTGACATGTTTTCTGAC-3′R<br>R 5′-TCACCCAACCACCCTGGTCTT-3′R |
| MDR1 | F 5′-CCCATCATTGCAATAGCAGG -3′R<br>R 5′-TGTTCAAACTTCTGCTCCTGA-3′R |
| GAPDH | F 5′- GAAGGTGAAGGTCGGAGTCA-3′R<br>R 5′- TTGAGGTCAATGAAGGGGTC-3′R |

Real-time PCR analysis of gene expression was done using the Rotor-Gene Q software package according to the manufacturer's guidelines (Qiagen). The relative level of RNA expression was normalized to GAPDH, and the difference in RNA expression was estimated using the 2-ΔΔCt method (20). which was expressed as the ratio between the expression of each gene. Triplicate measurements were done, and the average of all was analyzed in our results.

*2.6. Statistical Analysis.*

The experimental results are expressed as mean ± standard error of the mean (SEM). Data analysis was performed using the one-way ANOVA, and a p-value <0.05 was considered significantly different.

## 3. Results

*3.1. Evaluation of Drugs Cytotoxicity and Drugs synergism*

By analyzing the MTT cytotoxicity assay records, the cytotoxic order of our tested compounds on the MCF-7 cell line was as follows: DOX combined with NMD > DOX > NMD. The IC50 values of DOX combined with NMD and of DOX alone on the MCF-7 cell line were (28.93 and 1.66, respectively; Table 2). To further study whether NMD is affecting the cytotoxic potency of DOX, we carried out combination index analyses using the Chou Talalay equation and CompuSyn software (version 1.0; CompuSyn, Paramus, NJ, USA). Combined treatment of NMD and DOX yielded significantly greater growth inhibition in a dose-dependent manner.

The combination index (CI) was computed for the combination of NMD/DOX according to the method developed by Chou (21) to confirm and quantify the synergism observed with DOX and NMD. The two drugs were combined in a 1:25 ratio to calculate the CI value. Analysis shows a CI less than 1 corresponding to fraction affected (Fa) values from 0.5 to 0.95 which indicates a synergism between the two drugs in inhibiting the proliferation of MCF-7 cells (Table 2).

**Table 2: IC50 of Nimodipine and Doxorubicin as monotherapy or combination therapy in MCF-7 cells**

| Drug/Combo | CI | DOX | NMD |
|---|---|---|---|
| **DOX** | | 1.60±0.17 | |
| **NMD** | | | 28.93±1.25 |
| **Combination** | 0.97 | 0.58±0.04 | 17.54±0.37 |

We also calculated the DRI which represents the actual fold-change of dose attenuation in a synergistic combination at a given effect level compared with the drug alone. The Fa-DRI plot and Fa− log (DRI) plot demonstrates whether the influence of synergistic treatments may ameliorate side effects caused by cytotoxicity to normal cells. Figure (2D) demonstrates that the DRI of DOX values were higher than 1, which indicates favorable dose reduction when combined with NMD. Moreover, the mean DRI of DOX in the combination therapy was 3.01± 0.03, which suggests a three-fold dosage reduction compared to monotherapy.

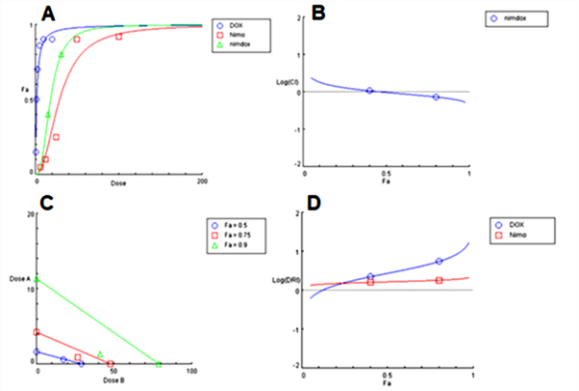


Fig. 2: The graphic representations obtained from the CompuSyn Report for NMD and DOX combinations (A) The combined inhibition effects of Nimodipine and Doxorubicin against the Mcf-7 cell line as analyzed with the CompuSyn system. (A) Dose-Effect Curve (B) Logarithmic Combination Index Plot (Log (CI)-Fa) (C) Isobologram for Combination, and (D) Logarithmic Dose Reduction Index Plot (Log (DRI)-Fa). Data are the average of three independent experiments ± SD.

### *3.2. Cell Cycle Analysis data*

Most anticancer agents act by arresting the cell cycle at definite stages of growth to exert their anticancer effect (22).

Flow cytometry is used in cell cycle analysis to distinguish cells at different phases of the cell cycle.

In this study, we tested the effect of the IC50 of DOX, NMD, and DOX/NMD combination to determine the definite phase at which cell cycle arrest takes place in the MCF-7 breast cancer cell line, and their impact on the cell population was recorded and compared to the control treated with solvent dimethyl sulfoxide (5% DMSO) as a vehicle (Figure 3).

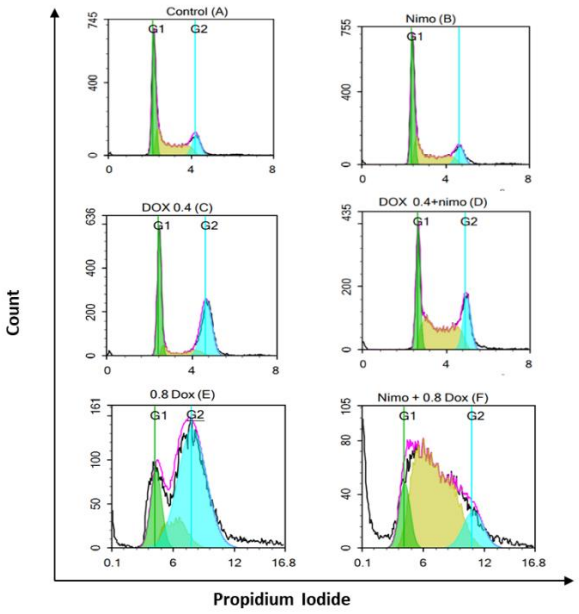


Fig. 3: Effects of doxorubicin alone or in combination with Nimodipine on Mcf-7 cells. Representative DNA cell cycle histograms.

Figure 4 compares the cell distribution percentage in each phase of the MCF-7 cell cycle 48 hours after each treatment. Findings showed that cells treated with DOX alone were arrested at the G2M phase, and cells treated with NMO were arrested at the S-phase phase, while cells treated with a combination of these two drugs were arrested at the S-phase in addition to G2/M phase. Moreover, combination therapy showed significant increase in pre-G1 apoptotic cells.

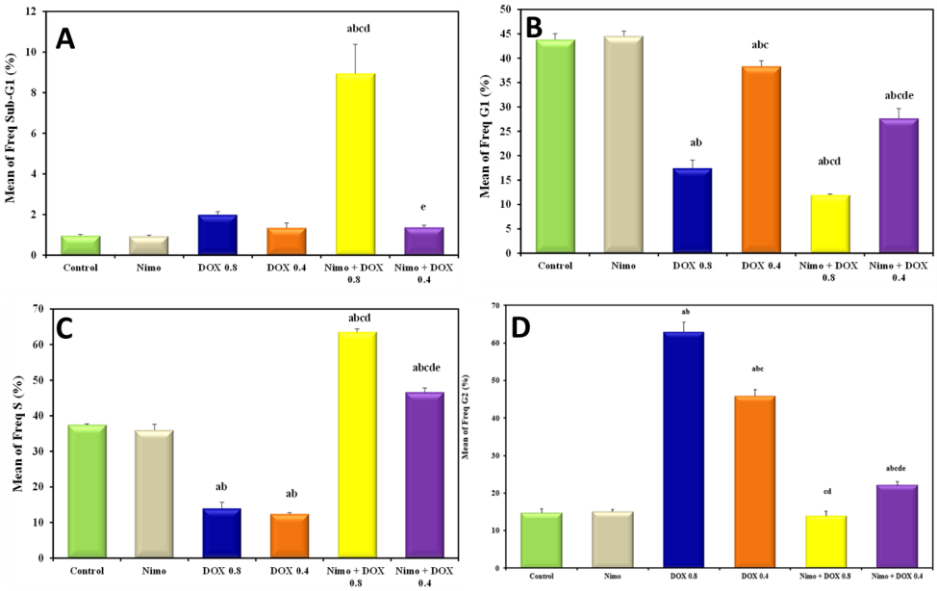


Figure 4: Impact of conjugates DOX, NMD, and DOX/NMD combination on the cell cycle phases of MCF-7 cells.

*3.3. Gene expression data.*

To unravel the underlying molecular mechanisms of the found synergistic antitumor effects of NMD and DOX on breast cancer, we studied the expression of apoptosis-related genes. Bcl2 is an antiapoptotic gene that reportedly negatively regulates the apoptosis pathway (22). In our results, Bcl2 expression was significantly decreased in NMD/DOX combination therapy compared with either monotherapy (Figure 5).

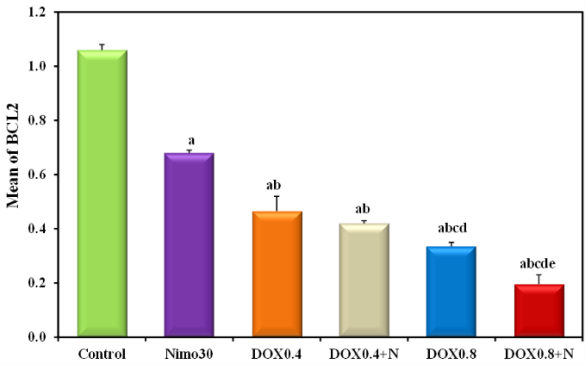


Fig. 5. Analysis of gene expression profiles of Bcl2 and MCF-7 cells treated with DOX, NMD, and DOX/NMD combination compared to blank by using real-time PCR. Different letters indicate significant differences between treatments ($p < 0.05$).

Contrary to Bcl2 gene expression, the proapoptotic gene Bax expression was found significantly increased in NMD/DOX combination therapy compared with either treatment alone (Figure 6).

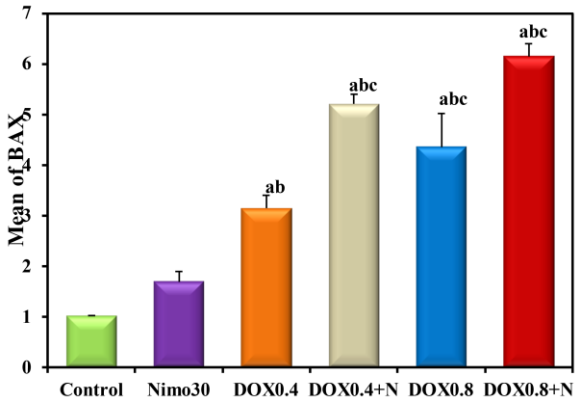


Fig. 6. Analysis of gene expression profiles of the multidrug resistance gene Bax in MCF-7 after treatment with DOX, NMD, and DOX/NMD combination compared to blank by using real-time PCR. Different letters indicate significant differences between treatments ($p < 0.05$).

Moreover, the multi-drug-resistance gene MDR1 is associated with the expected previously mentioned drug resistance, and its overexpression is observed in responses to some anticancer agents like DOX (23). Co-treatment of NMD and DOX decreased the expression of MDR1, and consequently increased the sensitivity of MCF-7 breast cancer to DOX (Figure 7), suggesting that adding NMD to DOX treatment protocols would decrease the therapeutic dose required for the treatment of Breast cancer and its toxic side effects as well.

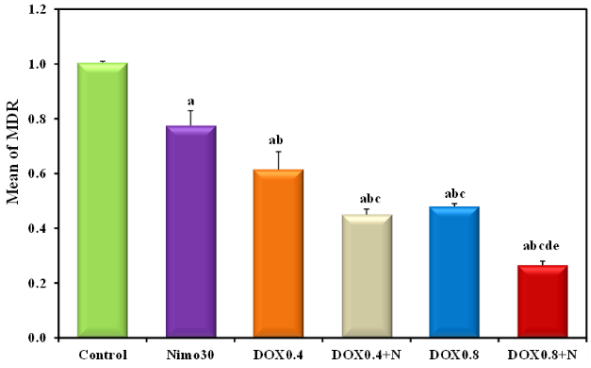


Fig. 7. Analysis of gene expression profiles of the multidrug resistance gene MDR1 in MCF-7 after treatment with DOX, NMD, and DOX/NMD combination compared to blank by using real-time PCR. Different letters indicate significant differences between treatments ($p < 0.05$).

## 4. Discussion

Drug resistance is a major problem in the management of patients with BC and has important implications for their poor prognosis (9). Currently, several strategies are suggested to reverse MDR and improve response to cancer treatments including the use of compounds or drugs that reverse the MDR-related P-gp such as the Ca2+ channel blocker verapamil and the immunosuppressant drug cyclosporine A (24,25). Although these drugs sensitize cancer cells to conventional Chemotherapy, they showed substantial adverse effects and pharmacokinetic problems (26) that hindered their clinical use. Therefore, a more potent compound to inhibit P-gp with fewer side effects is required in clinical settings. Gene expression profiling studies using DNA microarrays have indicated that treatment of BC with DOX alters the expression of a diverse group of genes in a time-dependent manner that could confer chemoresistance, including MDR1 (27).

Therefore, we aimed to investigate the potential of nimodipine (NMO, a calcium channel blocker drug) in sensitizing breast cancer cells to DOX and elucidate the underlying molecular mechanisms. Our results revealed that treatment of MCF-7 cells with either drug alone for 48 h induced distinct antiproliferative effects with IC50 values equivalent to 1.6 µM for DOX and 28.9 µM for NMD. However, when cells were treated with both drugs, the IC50 of DOX was significantly decreased to 0.53 µM in the combination therapy. This was confirmed by calculating the CI using CompuSyn software, which yielded values of CI <1. In addition, the isobologram curve showed that the

different concentrations of NMD and DOX which affect the same fraction (Fa) fall at the left of the predicted isobologram curve which confirms the synergistic interaction between the two drugs.

Moreover, the DRI values calculated using CompuSyn software indicated that the concentration of DOX required to inhibit 50% of MCF-7 cells could be reduced by a three-fold dosage compared to monotherapy when combined with NMD. These findings indicate that treatment of MCF-7 with a combination of DOX and NMD was able to induce a 3-fold reduction in the dose of DOX while maintaining comparable antiproliferative effects, in vitro and consequently lower its side effects. These findings agree with previously published reports on the sensitization of DOX by Empagliflozin (28) and Loperamide (29).

Arresting cell proliferation can be the result of cell death, cell-cycle arrest, or both. DOX is potent chemotherapy that exerts variable antitumor effects including intercalation into the DNA, resulting in cell cycle arrest either at the G1 or G2/M checkpoints to allow cell death (30). Furthermore, it has been reported that calcium channel proteins modulate the activity of specific cellular proteins which regulate the cell cycle progression and apoptosis (31).

In the current study, DNA content analysis of cells treated with the different regimes used revealed that the combined DOX/NMD treatment significantly reduced the proliferation index (sum of S-phase and G2M phase) of MCF-7 cells compared with cells treated with DOX alone. In addition, we found that the combined treatment DOX/NMD has significantly increased cells at the G1 phase of the cell cycle and increased pre-G0G1 apoptotic cells higher than that after DOX alone. These data suggest that the antiproliferative effects of this combination are due to both apoptosis induction and cell cycle arrest mechanisms significantly contributing to our cell cycle analysis findings. These data agree with the findings of Yokokura et al., (32) who demonstrated that treating multiple myeloma with calcium channel antagonists inhibits tumor proliferation in vivo and induced cell cycle arrest and apoptosis in vitro.

It has been proposed that overexpression of MDR-associated proteins contributes to the emergence of chemotherapy resistance to cancer (23). MDR1 gene is one of the multidrug genes that undergoes a rapid activation in tumors upon exposure to DOX, resulting in the emergence of chemoresistance (12).

It was reported that MDR1-related P-gp is modulated by calcium channel proteins (33) and that targeting it may sensitize tumors to chemotherapy via downregulating MDR1 (34,5). Following these data, our results indicate that the inclusion of the calcium channel-blocker NMD to DOX has significantly diminished MDR1 gene expression in Breast cancer MCF-7 cells. These data may confirm the role of the calcium channel blocker NMD in reversing the resistance of Breast Cancer to DOX via downregulating MDR1.

## 5. Conclusions

In summary, our data suggest that NMD could be a promising drug to be combined with DOX and repurposed against Breast cancer due to its chemo sensitization effect on DOX as proved by the reduction in the MDR1 gene and reflected by the enhanced cytotoxicity and apoptotic properties of DOX as indicated by the MTT and DNA cell cycle analyses. Moreover, the molecular mechanisms of these effects were revealed by the downregulation of the antiapoptotic gene Bcl-2, and the upregulation of the proapoptotic gene Bax. The chemo-sensitizing effect of the DOX/NMD combination will achieve two important outcomes. The first one will decrease the required therapeutic dose of DOX by approximately 3-folds and consequently decrease its toxic side effects, especially cardiotoxicity. Second, it will participate in the repression of DOX drug resistance, and resensitize tumor cells to DOX. Finally, we recommend further in vivo studies for NMD in combination with DOX as a new potential chemotherapeutic combination for treating Breast cancer.

## 6. Conflicts of interest

"There are no conflicts to declare".

## 7. Funding sources

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## 9. References


[1] Watson, I. R.; Takahashi, K.; Futreal, P. A.; Chin, L. Emerging Patterns of Somatic Mutations in Cancer. Nature Reviews Genetics, 14 (10), 703–718, 2013. https://doi.org/10.1038/nrg3539.

[2] Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R. L.; Torre, L. A.; Jemal, A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 68 (6), 394–424, 2018. https://doi.org/10.3322/CAAC.21492.

[3] Bertrand, K. A.; Tamimi, R. M.; Scott, C. G.; Jensen, M. R.; Pankratz, V. S.; Visscher, D.; Norman, A.; Couch, F.; Shepherd, J.; Fan, B.; Chen, Y. Y.; Ma, L.; Beck, A. H.; Cummings, S. R.; Kerlikowske, K.; Vachon, C. M. Mammographic Density and Risk of Breast Cancer by Age and Tumor Characteristics. Breast Cancer Research, 15 (6), 1–13, 2013. https://doi.org/10.1186/BCR3570/FIGURES/1.

[4] McGuire, S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press. Advances in Nutrition, 7 (2), 418–419, 2016. https://doi.org/10.3945/AN.116.012211.

[5] Cornwell, M. M.; Pastan, I.; Gottesman, M. M. Certain Calcium Channel Blockers Bind Specifically to Multidrug-Resistant Human KB Carcinoma Membrane Vesicles and Inhibit Drug Binding to P-Glycoprotein. Journal of Biological Chemistry, 262 (5), 2166–2170, 1987. https://doi.org/10.1016/S0021-9258(18)61633-3.

[6] Coleman, R. E.; Biganzoli, L.; Canney, P.; Dirix, L.; Mauriac, L.; Chollet, P.; Batter, V.; Ngalula-Kabanga, E.; Dittrich, C.; Piccart, M. A Randomised Phase II Study of Two Different Schedules of Pegylated Liposomal Doxorubicin in Metastatic Breast Cancer (EORTC-10993). European Journal of Cancer, 42 (7), 882–887, 2006. https://doi.org/10.1016/J.EJCA.2005.12.011.

[7] Gebbia, V.; Mauceri, G.; Fallica, G.; Borsellino, N.; Tirrito, M. L.; Testa, A.; Varvara, F.; Colombo, A.; Ferrera, P. Pegylated Liposomal Doxorubicin with Vinorelbine in Metastatic Breast Carcinoma. Oncology, 63 (1), 23–30, 2002. https://doi.org/10.1159/000065716.

[8] Thorn, C. F.; Oshiro, C.; Marsh, S.; Hernandez-Boussard, T.; McLeod, H.; Klein, T. E.; Altman, R. B. Doxorubicin Pathways: Pharmacodynamics and Adverse Effects. Pharmacogenetics and Genomics, 21 (7), 440, 2011. https://doi.org/10.1097/FPC.0B013E32833FFB56.

[9] Nedeljković, M.; Damjanović, A. Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge. Cells, 8 (9), 957, 2019. https://doi.org/10.3390/CELLS8090957.

[10] Pushpakom, S., Iorio, F., Eyers, P. et al. Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18, 41–58, 2019. https://doi.org/10.1038/nrd.2018.168

[11] Comerford, K. M.; Cummins, E. P.; Taylor, C. T. C-Jun NH2-Terminal Kinase Activation Contributes to Hypoxia-Inducible Factor 1α–Dependent P-Glycoprotein Expression in Hypoxia. Cancer Research, 64 (24), 9057–9061. https://doi.org/10.1158/0008-5472.CAN-04-1919.

[12] Abolhoda, A., Wilson, A. E., Ross, H., Danenberg, P. V., Burt, M., & Scotto, K. W. Rapid activation of MDR1 gene expression in human metastatic sarcoma after in vivo exposure to doxorubicin. Clinical Cancer Research, 5(11), 3352-3356, 1999.

[13] Park, K. A Review of Computational Drug Repurposing. Translational and Clinical Pharmacology, 27 (2), 59–63, 2019. https://doi.org/10.12793/TCP.2019.27.2.59.

[14] Polamreddy, P.; Gattu, N. The Drug Repurposing Landscape from 2012 to 2017: Evolution, Challenges, and Possible Solutions. Drug Discovery Today, 24 (3), 789–795, 2019. https://doi.org/10.1016/J.DRUDIS.2018.11.022.

[15] Canova, D.; Roatta, S.; Micieli, G.; Bosone, D. Cerebral Oxygenation and Haemodynamic Effects Induced by Nimodipine in Healthy Subjects. Functional Neurology, 27 (3), 169, 2012.

[16] Wang, S. D.; Herbette, L. G.; Rhodes, D. G. Structure of the Calcium Channel Antagonist, Nimodipine. urn:issn:0108-2701, 45 (11), 1748–1751, 1989. https://doi.org/10.1107/S010827018900329X.

[17] Hait, W. N.; Lazo, J. S. Calmodulin: A Potential Target for Cancer Chemotherapeutic Agents. https://doi.org/10.1200/JCO.1986.4.6.994, 4 (6), 994–1012, 2016. https://doi.org/10.1200/JCO.1986.4.6.994.

[18] Chou, T. C. Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies. Pharmacological Reviews, 58 (3), 621–681, 2006. https://doi.org/10.1124/PR.58.3.10.

[19] Osman, A. M. M.; Bayoumi, H. M.; Al-Harthi, S. E.; Damanhouri, Z. A.; ElShal, M. F. Modulation of Doxorubicin Cytotoxicity by Resveratrol in a Human Breast Cancer Cell Line. Cancer Cell International, 12 (1), 1–8, 2012. https://doi.org/10.1186/1475-2867-12-47/TABLES/3.

[20] Livak, K. J.; Schmittgen, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods, 25 (4), 402–408, 2001. https://doi.org/10.1006/METH.2001.1262.

[21] Chou, T. C. Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. Cancer Research, 70 (2), 440–446, 2010. https://doi.org/10.1158/0008-5472.CAN-09-1947/655517/P/DRUG-COMBINATION-STUDIES-AND-THEIR-SYNERGY.

[22] Asnaghi, L.; Calastretti, A.; Bevilacqua, A.; D'Agnano, I.; Gatti, G.; Canti, G.; Delia, D.; Capaccioli, S.; Nicolin, A. Bcl-2 Phosphorylation and Apoptosis Activated by Damaged Microtubules Require MTOR and Are Regulated by Akt. Oncogene 2004 23:34, 23 (34), 5781–

5791, 2004. https://doi.org/10.1038/sj.onc.1207698.
[23] Tada, Y.; Wada, M.; Migita, T.; Nagayama, J.; Hinoshita, E.; Mochida, Y.; Maehara, Y.; Tsuneyoshi, M.; Kuwano, M.; Naito, S. Increased Expression of Multidrug Resistance-Associated Proteins in Bladder Cancer during Clinical Course and Drug Resistance to Doxorubicin. International Journal of Cancer, 98 (4), 630–635, 2002. https://doi.org/10.1002/IJC.10246.
[24] Lampidis, T. J.; Krishan, A.; Planas, L.; Tapiero, H. Reversal of Intrinsic Resistance to Adriamycin in Normal Cells by Verapamil. https://home.liebertpub.com/cdd, 3 (4), 251–259, 2009. https://doi.org/10.1089/CDD.1986.3.251.
[25] Hoof, T.; Demmer, A.; Christians, U.; Tümmler, B. Reversal of Multidrug Resistance in Chinese Hamster Ovary Cells by the Immunosuppressive Agent Rapamycin. European Journal of Pharmacology: Molecular Pharmacology, 246 (1), 53–58, 1993. https://doi.org/10.1016/0922-4106(93)90009-X.
[26] Coley, H. M. Overcoming Multidrug Resistance in Cancer: Clinical Studies of p-Glycoprotein Inhibitors. Methods Mol Biol, 596, 341–358, 2010. https://doi.org/10.1007/978-1-60761-416-6_15/TABLES/1.
[27] Kudoh, K., Ramanna, M., Ravatn, R., Elkahloun, A. G., Bittner, M. L., Meltzer, P. S., ... & Chin, K. V. Monitoring the expression profiles of doxorubicin-induced and doxorubicin-resistant cancer cells by cDNA microarray. Cancer research, 60(15), 4161-4166, 2000.
[28] Eliaa, S. G.; Al-Karmalawy, A. A.; Saleh, R. M.; Elshal, M. F. Empagliflozin and Doxorubicin Synergistically Inhibit the Survival of Triple-Negative Breast Cancer Cells via Interfering with the MTOR Pathway and Inhibition of Calmodulin: In Vitro and Molecular Docking Studies. ACS Pharmacology and Translational Science, 3 (6), 1330–1338, 2020. https://doi.org/10.1021/ACSPTSCI.0C00144/SUPPL_FILE/PT0C00144_SI_001.PDF.
[29] Elia, S. G.; Al-Karmalawy, A. A.; Nasr, M. Y.; Elshal, M. F. Loperamide Potentiates Doxorubicin Sensitivity in Triple-Negative Breast Cancer Cells by Targeting MDR1 and JNK and Suppressing MTOR and Bcl-2: In Vitro and Molecular Docking Study. Journal of Biochemical and Molecular Toxicology, 36 (1), e22938, 2022. https://doi.org/10.1002/JBT.22938.
[30] Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G.; Gianni, L. Anthracyclines: Molecular Advances and Pharmacologic Developments in Antitumor Activity and Cardiotoxicity. Pharmacological Reviews, 56 (2), 185–229, 2004. https://doi.org/10.1124/PR.56.2.6.
[31] Berchtold, M. W.; Villalobo, A. The Many Faces of Calmodulin in Cell Proliferation, Programmed Cell Death, Autophagy, and Cancer. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1843 (2), 398–435, 2014. https://doi.org/10.1016/J.BBAMCR.2013.10.021.
[32] Yokokura, S.; Yurimoto, S.; Matsuoka, A.; Imataki, O.; Dobashi, H.; Bandoh, S.; Matsunaga, T. Calmodulin Antagonists Induce Cell Cycle Arrest and Apoptosis in Vitro and Inhibit Tumor Growth in Vivo in Human Multiple Myeloma. BMC Cancer, 14 (1), 1–11, 2014. https://doi.org/10.1186/1471-2407-14-882/FIGURES/7.
[33] Schlemmer, S. R.; Yang, C. H.; Sirotnak, F. M. Functional Modulation of Multidrug Resistance-Related P-Glycoprotein by Ca2+-Calmodulin *. Journal of Biological Chemistry, 270 (19), 11040–11042, 1995. https://doi.org/10.1074/JBC.270.19.11040.
[34] Luo, L.; Sun, Y. J.; Yang, L.; Huang, S.; Wu, Y. J. Avermectin Induces P-Glycoprotein Expression in S2 Cells via the Calcium/Calmodulin/NF-KB Pathway. Chemico-Biological Interactions, 203 (2), 430–439, 2013. https://doi.org/10.1016/J.CBI.2013.03.009.